

EFFECTS OF PORCINE ZONA PELLUCIDA IMMUNOCONTRACEPTIVES IN ZOO FELIDS

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Abstract: Methods of contraception are necessary for management of zoo felids; however, the most commonly used contraceptive (melengestrol acetate implant) is associated with serious adverse reactions with long-term use. Porcine zona pellucida (pZP) vaccines are promising as contraceptives, but their safety in zoo felids has not been tested. pZP vaccine was administered to 27 female felids representing 10 species, including African lion (*Panthera leo*), Asian leopard (*P. pardus*), jaguar (*P. onca*), tiger (*P. tigris*), snow leopard (*P. uncia*), cougar (*Felis concolor*), Siberian lynx (*F. lynx*), Canada lynx (*F. canadensis*), serval (*F. serval*), and bobcat (*F. rufus*), in 15 facilities. Over 6 wk, each animal received three i.m. injections of 65 µg pZP with Freund's complete adjuvant (FCA), Freund's incomplete adjuvant, or carbopol as the adjuvant. Behavioral signs of estrus were seen in 14 of the vaccinated felids. An unacceptably high incidence of adverse reactions was seen including injection site swelling, lameness, limb swelling, or abscessation (or all) in five felids after injection with FCA as the initial adjuvant. Adverse behavioral signs, including increased irritability and aggression, were seen in four felids. Six of the felids were assayed for antibodies against pZP during the 12 mo after vaccination; all showed antibody production. Antibody levels appeared to peak 1–4 mo after vaccination began, although elevated antibody levels persisted in two animals for >12 mo after the first injection. All vaccinated felids were ovariectomized 3–13 mo after vaccination. Folliculogenesis was present in all treated animals, and there was no histopathologic evidence of inflammatory damage to ovaries. Contraceptive efficacy was not specifically evaluated in this study; however, two of the three felids housed with an intact male became pregnant during the study, one of which gave birth to healthy cubs.

Key words: *Panthera* sp., *Felis* sp., contraception, Freund's adjuvant, immunocontraception, zona pellucida.

INTRODUCTION

Contraception is routinely used in zoo felids to prevent overpopulation and allow preservation of genetic variability within captive populations.⁵² Permanent contraception is safely achieved by surgical sterilization. The genetic priorities of endangered species breeding programs necessitate conservation of maximum genetic resources, so temporary contraceptive methods are preferable. Melengestrol acetate-impregnated silastic implants are currently recommended as the most reliable means of reversible contraception for zoo felids, according to the Contraceptive Advisory Group of the Amer-

ican Zoo and Aquarium Association (AZACAG).⁷ Long-term or repeated use of these implants has been associated with endometrial hyperplasia, endometrial mineralization, and uterine and mammary cancer.^{22,46,47} The use of such other progestins as levonorgestrel and medroxyprogesterone acetate, androgens as mibolerone,¹⁵ and surgical methods as vas deferens occlusion has been investigated in zoo felids. None has provided reliable contraception, patient safety, ease of administration, and reversibility.

Vaccination using zona pellucida (ZP) antigen shows promise for reversible contraception in many species. Reproductive immunologists continue to attempt to develop a ZP vaccine that could be administered easily to large numbers of women in rapidly growing countries, stemming population growth with minimal ethical concerns and completely reversible contraceptive effects.^{2,3,5,8,12,20,29,39,40,48,54} Porcine-derived ZP (pZP) vaccination has become a valuable population control measure for wild ungulates.^{13,16,24,31–34,43–56} ZP immunocontraception has been performed effectively in a small number of African lions and a cougar (Kirkpatrick, unpubl. data) but with poor efficacy in domestic cats.^{17,25}

ZP glycoproteins are found in oocytes at all stages of folliculogenesis, even as early as the primordial or primary follicle stage.¹⁸ During normal fertilization, sperm attach to receptor glycoproteins in the ZP matrix on the outside of an ovulated ovum,

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thus inducing the acrosome reaction in a spermatozoon and allowing the single spermatozoon to complete fertilization.¹⁰ If ZP antibodies are present in follicular fluid before ovulation, the resultant antibody-antigen complex prevents sperm penetration of the ZP matrix primarily by steric hindrance (physical occlusion of sperm receptor sites) and thus prevents the cascade of events that lead to fertilization of the oocyte. In some species, the immune response to ZP vaccination includes cell-mediated as well as humoral components, and T-cell involvement has caused lymphocytic inflammation in all stages of follicles in ZP-vaccinated mice.^{38,49} Previous studies have reported oophoritis, oocyte depletion, disruption of follicular development, and ovarian dysfunction in ZP-vaccinated nonhuman primates, mice, rabbits, and dogs,^{3,9,26,30,41,50,53,57,58} although it is not clear whether these effects were due to the ZP doses used, purity of ZP antigen, or other factors.

ZP proteins show similarity between taxonomic groups,^{23,42} therefore porcine ovaries (readily available from abattoirs) have been used as the basis for most ZP vaccines. However, domestic cat ZP and pZP share few epitopes, and this has been cited as the reason for lack of contraceptive efficacy in pZP-vaccinated domestic cats, despite high levels of pZP antibodies being present.²⁷ Similar epitope research has not been performed in other felid species, and it is uncertain whether pZP antibodies effectively block sperm binding sites in nondomestic felids.

High immunogenicity and antibody production have been achieved with ZP vaccines adjuvanted with Freund's complete adjuvant (FCA).⁴ However, FCA has been associated with adverse reactions, including local and systemic granulomatous inflammation, due to mycobacterial cell wall and paraffin oil components.^{6,36,60} Adverse reactions have also been seen in domestic cats (Munson, unpubl. data). Mycobacterial components in FCA cause tuberculin test positivity in animals that have had FCA administered. Freund's incomplete adjuvant (FIA) does not contain mycobacterial components and is therefore less likely to cause granulomatous injection site reactions. FIA is also less immunogenic than FCA. Carbopol is a water-soluble high-molecular weight carbomer that has been used as an adjuvant in pigs and rabbits without adverse effects.^{14,19}

Research has recently focused on ways to minimize the adverse effects of ZP vaccination by using less immunogenic adjuvants and more purified and specific (recombinant subunit) forms of ZP antigen protein.^{1,28,35,37,51,55,57} However, the decrease in

broad-spectrum immunogenicity results in decreased contraceptive efficacy, which is problematic for population management of zoo species.

We administered partially purified adjuvanted pZP to 27 zoo felids representing 10 species, followed them clinically, and then evaluated their ovaries histopathologically up to 14 mo after vaccination to determine whether ZP immunocontraceptives had adverse effects in these species. Serum ZP antibodies were also measured in six of the felids to evaluate the magnitude and duration of their humoral response.

MATERIALS AND METHODS

Study animals

North American institutions housing nondomestic felids were contacted to recruit permanently surplus (not intended for breeding) females for this study. Twelve African lions (*Panthera leo*), six cougars (*Felis concolor*), two tigers (*P. tigris*), one jaguar (*P. onca*), one Asian leopard (*P. pardus*), one snow leopard (*P. uncia*), one serval (*F. serval*), one Siberian lynx (*F. lynx*), one Canada lynx (*F. canadensis*), and one bobcat (*F. rufus*) were enrolled in the study (Table 1). A control group of nonvaccinated felids was chosen from the AZA-CAG's disease surveillance database to match the study group animals as closely as possible regarding age, previous contraceptive history, and species. All vaccinated felids were sexually mature at the time of first pZP vaccination, and their ages ranged from 2 to 18 yr at time of ovariectomy. This study was not designed as a contraceptive efficacy trial; institutions were advised that vaccinated felids should not be considered contracepted and should not be allowed to breed during the study. However, separation from males was not always feasible, and three felids were housed with sexually intact males during the study.

Vaccine and adjuvant preparation

pZP were isolated using techniques described previously.^{11,21} Frozen, thawed porcine ovaries were minced in cold phosphate-buffered saline (PBS) using a ganged razor-blade apparatus. The oocytes were separated from other tissues, including granulosa cells, by screen filtration, counted, and homogenized. The zonae were isolated on a 48- μ m screen, heat-solubilized at 70°C, and frozen in doses of 65 μ g protein (approximately 5,000 zonae) in 0.5 ml PBS until shipment and use by participating institutions.

Three adjuvants were used for preparation of pZP vaccine: FCA (F5506, Sigma-Aldrich Co., St.

Table 1. Descriptive data for 27 female zoo felids vaccinated with porcine zona pellucida, including 12 lions (*Panthera leo*), six cougar (*Felis concolor*), two tigers (*P. tigris*), one jaguar (*P. onca*), one Asian leopard (*P. pardus*), one snow leopard (*P. uncia*), one serval (*F. serval*), one Siberian lynx (*F. lynx*), one Canada lynx (*F. canadensis*), and one bobcat (*F. rufus*).^a

Animal	Age (yr)	Recent MGA exposure ^b	Adjuvants	Months until OVH	Estrus seen?	Comments
Lion 1	9	no	Carb/Carb/Carb	6	yes	NAR ^c
Lion 2	9	yes	Carb/Carb/Carb	7	ND	NAR
Lion 3	11	no	Carb/Carb/Carb	13	yes	NAR
Lion 4	11	no	Carb/Carb/Carb	12	yes	NAR
Leopard	14	no	Carb/Carb/Carb	6	yes	NAR
Tiger 1	9	no	Carb/Carb/Carb	12	ND	NAR, pregnancy
Tiger 2	9	no	Carb/Carb/Carb	3	ND	NAR
Jaguar	14	no	Carb/Carb/Carb	7	ND	NAR
Lion 5	15	yes	FCA/FIA/FIA	3	ND	injection site swelling
Lion 6	6	no	FCA/FIA/FIA	3	yes	NAR
Lion 7	5	yes	FCA/FIA/FIA	6	ND	NAR
Lion 8	8	no	FCA/FIA/FIA	12	yes	behavior change, limb swelling
Lion 9	8	no	FCA/FIA/FIA	12	yes	behavior change, injection site swellings
Lion 10 ^d	8	no	FCA/FIA/no vaccination	6	yes	behavior change, cellulitis, fistulous tracts
Cougar 1	2	no	FCA/FIA/FIA	4	yes	NAR
Cougar 2	3	no	FCA/FIA/FIA	14	no	NAR
Cougar 3	7	no	FCA/FIA/FIA	12	yes	NAR
Cougar 4	7	no	FCA/FIA/FIA	12	no	NAR
Cougar 5	9	yes	FCA/FIA/FIA	12	no	behavior change
Siberian lynx	10	no	FCA/FIA/FIA	6	no	lameness
Canada lynx	6	yes	FCA/FIA/FIA	8	no	NAR, pregnancy
Serval	11	no	FCA/FIA/FIA	12	yes	NAR
Bobcat	6	yes	FCA/FCA/FCA	13	yes	NAR
Cougar 6	10	no	FCA/FCA/FCA	6	yes	NAR
Lion 11	18	yes	FIA/FIA/FIA	6	ND	NAR
Lion 12	12	yes	FIA/FIA/FIA	6	yes	NAR
Snow leopard	5	yes	FIA/FIA/FIA	6	ND	NAR

^a FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; Carb, carbopol; MGA, melengestrol acetate; OVH, ovario hysterectomy; ND, not definitely known.

^b MGA impregnated in silastic, placed i.m. or s.c. as a contraceptive implant. Recent MGA exposure was defined as less than 2 yr between MGA implant removal and OVH.

^c NAR, no adverse reactions reported by clinical veterinarian.

^d Lion 10 did not receive a third vaccination.

Louis, Missouri 63178, USA), FIA (F5881, Sigma-Aldrich Co.), and carbopol (Carbopol 934, B. F. Goodrich, Cleveland, Ohio 44141, USA). Individual vaccines were prepared using 0.5 ml pZP and 0.5 ml adjuvant, emulsified together immediately before injection.

Study schedule

pZP vaccine was administered i.m. by hand injection, pole syringe, Capchur[®] dart (Palmer Chemical and Equipment Co., Douglasville, Georgia 30133, USA), Telinject[®] dart (Telinject, Saugus, California 91350, USA), or Pneu-dart[®] dart (Pneu-

dart, Williamsport, Pennsylvania 17703, USA) in a 1-ml volume. Each felid received three inoculations over a 1.5-mo period, except one lion that did not receive a third inoculation because of injection site reactions seen after the first two inoculations (Table 1). Table 1 also shows the order of adjuvants used for the three injections in each individual. One bobcat and one cougar received FCA as the vaccine adjuvant for all three inoculations, instead of receiving FIA in the second and third inoculations as had been intended. One snow leopard and two lions received FIA as the vaccine adjuvant for all three inoculations. In total, 19 felids received FCA or

FIA (or both) as their vaccine adjuvant (pZP-F), and eight felids received carbopol as their vaccine adjuvant (pZP-C). Behavior changes, subjective signs of estrus (vocalization, posturing, and other signs decided upon by each institution), and clinical signs of illness were recorded at each institution during the course of the study. Blood was collected from six felids before initial pZP vaccination and then at approximately 1, 3, 6, 8, 10, and 12 mo after initial vaccination, although not all time points were achieved for all animals. Ovariectomy was performed on all vaccinates at approximately 3, 6, or 12 mo after initial pZP injection. The uterus and ovaries were fixed in 10% neutral buffered formalin and shipped to the University of Tennessee for histopathologic examination.

Antibody analysis

Serum was harvested and frozen until it was shipped to the University of California for antibody measurement. Antizona antibody analysis was accomplished by the enzyme-linked immunosorbent assay (ELISA).⁵⁹ Fifty microliters of 5 µg/ml zona antigen solution in 0.1 M glycine buffer (pH 9.5) was placed in each well of a flat-bottom ELISA microplate ("Sumilon" low protein binding, Cat. #MS-3496, E & K Scientific Products, Saratoga, California 95070, USA) and incubated overnight at 4°C. At room temperature (20–22°C), the plate was washed once and incubated with 200 µl PBS-Tween for 30 min to block unspecific binding sites. After two more PBS-Tween washes, the treatment of the plate consisted of subsequent incubations with 50 µl/well of TBS-Tween-diluted reagents used in the following order with three washes each in between: study felid's serum diluted 1:500, biotinylated goat anti-cat IgG diluted 1:250 (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland 20879, USA), and alkaline phosphatase avidin diluted 1:1,000 (Zymed Laboratories, San Francisco, California 94101, USA). Finally, 50 µl of substrate solution of 1 mg *p*-nitrophenyl phosphate/ml (5-mg tablets, Sigma-Aldrich Co.) in 10% diethanolamine buffer (pH 9.8) was distributed to each well. Reference serum consisting of pooled sera with antizona antibody levels of a medium-positive range (compared with anti-pZP antibodies generated from pZP-injected domestic cats) was used as a positive control. Pooled preimmunization sera served as negative controls. The plate was scanned for absorbance at 405 nm using an MR 580 Microelisa Auto Reader (Dynatech Laboratories, Alexandria, Virginia 22313, USA) when the absorbance of the positive reference serum had reached a level close to 0.8 after incubation of approxi-

mately 10 min. The experimental sera were scanned in duplicate, and their values were expressed as optical densities.

Histopathologic examination

Both ovaries from each felid were examined for gross lesions, sectioned in a sagittal plane, then one half of each ovary was sectioned at 5-mm increments in a transverse plane. Tissues were embedded in paraffin, sectioned at 7 µm, and stained with hematoxylin and eosin.

All ovarian sections were examined for evidence of follicular development and lesions. The following features were quantified: primordial and primary follicles, secondary follicles with oocytes but no visible zonae, secondary follicles with visible zona development, tertiary follicles, corpora lutea (CL), granulosa cell nests, and atretic secondary or tertiary follicles. Presence or absence of inflammatory cells, mineralization, or neoplasia was assessed. The character and degree of inflammation were recorded.

Ovaries from 10 animals (five controls, two pZP-F, and three pZP-C) were immunostained to confirm the presence of lymphocytes. For these procedures, slides were deparaffinized with xylene and rehydrated through graded alcohols. Sections were steamed at 94°C for 30 min in citrate buffer, pH 6.6, for antigen retrieval. Endogenous peroxidase was blocked with Peroxidase Blocking Reagent (Dako Corporation, Carpinteria, California 93013, USA) for 5 min at room temperature. Then, slides were incubated with prediluted primary monoclonal antibodies against CD3 (T cells, Novocastra Laboratories Ltd., Newcastle on Tyne, U.K.) or CD79a (B cells, Dako Corporation) for 30 min at room temperature. Domestic cat spleen was used as a positive control. Negative control slides were incubated without primary antibodies. Slides were incubated with secondary biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, California 94010, USA) for 30 min, followed by streptavidin-horseradish peroxidase (Zymed) for 20 min following the manufacturer's protocols. Amino-9-ethyl-carbazole (Sigma-Aldrich Co.) was used as the chromagen. Slides were counterstained with hematoxylin (Gill's formula, Fisher Scientific, Pittsburgh, Pennsylvania 15275, USA).

Statistical analyses

For quantitative ovarian analyses, the mean number of each follicular stage was compared between pZP-treated and control animals by nonparametric tests (Mann-Whitney test). Differences among the two adjuvant groups and controls also were as-

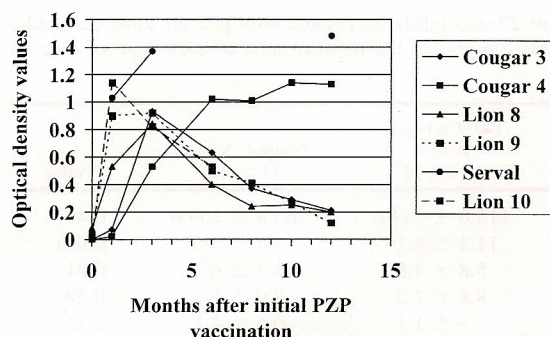


Figure 1. Pre- and postinoculation levels of antibodies against porcine zona pellucida (pZP) in six pZP-vaccinated female zoo felids, including three lions (*Panthera leo*), two cougars (*Felis concolor*), and one serval (*F. serval*). Antibody levels were measured by enzyme-linked immunosorbent assay and are expressed as optical density values, which are proportional to the amount of antibody present. Animals were vaccinated at time 0, 0.75, and 1.5 mo, except for lion 10, which was vaccinated only once, at time 0.

essed using nonparametric analysis of variance (Kruskal-Wallis test). For the Freund's adjuvant group, ovarian features were compared among the 3, 6, and 9 mo postvaccination groups. Qualitative ovarian data including presence or absence of zona, CL, inflammation, mineralization, or neoplasia were compared between pZP treatment groups and between vaccinated versus nonvaccinated felids in contingency tables. For all analyses, the significance level was set at $P < 0.05$.

RESULTS

Immunocontraceptive response

Antibodies to pZP were measurable in all six felids from which sera were collected (Fig. 1). Three of these felids had received pZP with a FCA/FIA/FIA adjuvant protocol, one had received pZP with a FCA/FIA adjuvant protocol (no third vaccination), and two had received pZP with a FIA/FIA/FIA adjuvant protocol. The highest optical density measured in each felid was $\geq 84\%$ that of positive reference serum (range 84–148%). Highest antibody levels were measured at 1 mo after the first vaccination in one felid, at 3 mo in three felids, and at 10 or 12 mo in two felids. Ovaries of felids that had persistently high antibody levels (serval and cougar 4) appeared histologically similar to those of felids with lower antibody levels, with no evidence of impeded folliculogenesis, follicular damage, or increased inflammation. Two of the three felids housed with males became pregnant during

the study; these felids' sera were not available for antibody measurement.

Adverse reactions and behavioral signs

Clinically apparent adverse reactions were seen in several of the felids vaccinated with FCA as the initial adjuvant but not in felids receiving other adjuvants (Table 1). Injection site swellings were seen in four felids for at least 3 mo after their second vaccinations. Injection site reactions did not appear to be associated with a particular injection method or institution because they occurred after pZP had been hand-injected or darted and at more than one facility. Two lions from separate facilities developed firm nodular masses at the site of pZP injection. One of these masses was biopsied within 3 mo of development, and biopsy revealed an extensive pyogranulomatous reaction at the site. Admixed with the abundant epithelioid macrophages and neutrophils were clear vacuoles that are characteristic of paraffin oil from Freund's adjuvant. In another lion, an injection site reaction progressed to cellulitis of both hind limbs with fistulous draining tracts that eventually healed as scars during the subsequent year. In another lion from the same facility, an injection site reaction involved swelling of one entire hind limb after the third vaccination. One lynx showed transient lameness without apparent injection site swelling for 3 days after second vaccination. Abnormal behavior was seen in four felids after vaccination, including increased irritability, "masculine" behavior, and aggression for the entire duration of the study. Behavioral signs of estrus were seen in at least 14 of the vaccinated felids (Table 1), and breeding was often observed in felids housed with intact or vasectomized males.

Ovarian and uterine histopathology

All animals had evidence of ongoing folliculogenesis and many had CL, indicating recent ovulation. Quantitative findings of follicular development are presented in Table 2. Control animals had fewer developing secondary follicles and more atretic secondary follicles than animals treated with pZP-F (FCA and FIA together). Animals vaccinated with pZP-C had significantly more secondary follicles without zona than either pZP-F or controls. All animals except two controls and one pZP-F had plentiful healthy zona. Minimal lymphocytic infiltrates composed of small numbers of B- and T cells were present in the stroma and atretic follicles of most animals including 20 of 25 controls, 14 of 19 pZP-F-treated animals, and all pZP-C-treated animals ($P = 0.7$). There was no qualitative difference in degree or distribution of inflammation

Table 2. Quantitative and qualitative features in ovaries of 27 zoo felids vaccinated with porcine zona pellucida. *N* refers to the number of felids in each treatment group. Table entries list the number seen of each type of ovarian feature.^a

Ovarian feature	PZP-Freund's, <i>N</i> = 19 ($\bar{x} \pm SD$)	PZP-Carbopol, <i>N</i> = 8 ($\bar{x} \pm SD$)	Control, <i>N</i> = 25 ($\bar{x} \pm SD$)	<i>P</i> value
Primordial and primary follicles	169.6 \pm 141.9	115.6 \pm 144.6	165.8 \pm 159.6	0.46
Secondary follicle with no zona	7.7 \pm 6.5 ^b	11.3 \pm 8.1 ^c	3.3 \pm 3.0 ^b	0.001
Secondary follicle with zona	15.1 \pm 12.4 ^b	5.8 \pm 4.8	6.7 \pm 6.2 ^c	0.01
Tertiary or preovulatory	13.8 \pm 11.7	8.8 \pm 7.2	8.9 \pm 7.2	0.29
Corpora lutea	1.1 \pm 1.4	1 \pm 1.1	0.6 \pm 1.1	0.15
Granulosa cell nests	0.5 \pm 1.7	0.5 \pm 0.5	1.0 \pm 2.0	0.26
Atretic secondary follicles	1.4 \pm 1.3 ^b	1.9 \pm 1.6	6.5 \pm 6.3 ^c	0.0004
Atretic tertiary follicles	15.8 \pm 7.2	13.8 \pm 9.3	17.0 \pm 10.6	0.69

^a Within rows, values with different superscripts are significantly different ($P < 0.05$) by Kruskal-Wallis nonparametric tests.

among treatment groups. One pZP-C-treated jaguar with an ovarian papillary cystadenocarcinoma had abundant lymphocytes infiltrating the theca and surrounding zonae. There were no significant differences in parameters among ovaries obtained 3, 6, or 12 mo after pZP-F vaccination. Moderate to severe cystic endometrial hyperplasia was present in 66% of both pZP-vaccinated and control groups ($P = 1.24$).

DISCUSSION

This study showed that pZP vaccination induces an immunologic response in zoo felids, but that vaccination is not necessarily efficacious. Folliculogenesis and ovulation continued in the felids of this study, despite the presence of ZP antibodies. These data are consistent with findings of a previous pZP clinical trial in African lions (Kirkpatrick, unpubl. data), in which pseudopregnancies were seen.

No ovarian damage was noted in any of the study animals. Differences among treatment groups for quantitative ovarian parameters were not considered biologically relevant because all stages of folliculogenesis were plentiful. Unlike reports of immune-mediated damage to ovaries in ZP-vaccinated dogs, rodents, rabbits, and primates, no significant inflammation was observed in the ovaries of study felids. The prominent lymphocytic infiltrates in the pZP-C-treated jaguar with ovarian cancer were likely in response to the tumor. It is possible that the inflammatory lesions in other studies were due to adjuvant effects or lack of purity of the ZP antigen.

Although previous studies involving wildlife species have suggested correlation between pZP antibody levels and contraceptive efficacy, these findings may not be applicable to felids.^{17,25} In our

study, neither pZP-F nor pZP-C vaccines were 100% effective in preventing conception and pregnancy. The two felids that became pregnant in our study did not have sera collected for antibody measurement, so it was not possible to correlate antibody levels to contraception. These two animals may have had low antibody levels, so that ova were not effectively coated with antibody and therefore not protected from fertilization. Alternatively, the contraceptive effects of ZP vaccination may be due in part to cell-mediated immunity. However, the ovaries of the ZP-vaccinated animals of our study did not contain significantly more lymphocytes than control animals and those lymphocytes present were randomly distributed (not spatially associated with follicles). These findings suggest that cell-mediated immune damage did not occur in ZP-vaccinated zoo felids. It is possible that zoo felids' ZP epitopes differ significantly from pZP epitopes, therefore making pZP antibodies ineffective in blocking ZP sites on the zonae of zoo felids, as has been described in domestic cats.²⁷ Further research regarding epitope characteristics of porcine and zoo felid ZP and antibody cross-reactivity will be necessary to understand the contraceptive potential of pZP antibodies in zoo felids.

The Freund's-adjuvanted pZP vaccine cannot be recommended for zoo felids because six of the 16 felids given FCA as one of the vaccine adjuvants experienced significant adverse reactions. Tissue changes were characterized by a marked granulomatous reaction, which was likely due to both the *Mycobacterium tuberculosis* antigens and the paraffin lipid in FCA. Behavioral reactions including irritability and dominant behavior may have been due to pain associated with vaccination site reactions and resultant defensive responses toward caretakers. The mycobacterial antigens and paraffin lip-

id of FCA induce a marked immunologic response and make FCA an effective adjuvant. It is not known whether Freund's modified adjuvant containing *M. butyricum* would induce similar reactions in zoo felids or be equally immunogenic. Carbopol-adjuvanted pZP vaccination did not induce adverse reactions in this study.

CONCLUSIONS

Freund's-adjuvanted pZP contraceptive vaccines used in this study were associated with an unacceptably high incidence of adverse vaccine reactions in zoo felids, and the cause of these reactions was likely FCA. pZP vaccination regardless of the adjuvant was not associated with ovarian lesions in this study. Pregnancy and successful parturition occurred despite pZP vaccination in some felids. Until safer, effective adjuvants are available and ZP epitopes can be designed that incite an effective immune response, pZP vaccination should not be used for contraception of zoo felids.

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